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(71) Applicant *(for all designated States except US)*: THE NORWEGIAN RADIUM HOSPITAL RESEARCH FOUNDATION [NO/NO]; Montebello, N-0310 Oslo 3 (NO).

(71) Applicant *(for GB only)*: DZIEGLEWSKA, Hanna, Eva [GB/GB]; Frank B Dehn & Co., Imperial House, 15/19 Kingsway, London WC2B 6UZ (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GIERSKCKY, Karl, Erik [NO/NO]; The Norwegian Radium Hospital, Montebello, N-0310 Oslo (NO). MALIK, Zvi [IL/IL]; Bar Ilan University, Life Sciences Dept., 52900 Ramat-Gan (IL). MOAN, Johan [NO/NO]; Institute of Cancer Research, Montebello, N-0310 Oslo (NO). PENG, Qian [CN/NO]; Institute of Cancer Research, Montebello, N-0310 Oslo (NO). STEEN, Harald [NO/NO]; Institute of Cancer Research, Montebello, N-0310 Oslo (NO). WARLOE, Trond [NO/NO]; The Norwegian Radium Hospital, Montebello, N-0310 Oslo (NO).

(74) Agents: DZIEGLEWSKA, Hanna, Eva et al.; Frank B Dehn & Co, Imperial House, 15/19 Kingsway, London WC2B 6UZ (GB).

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(54) Title: PHOTOCHEMOTHERAPEUTIC COMPOSITIONS CONTAINING 5-AMINOLEVULINIC ACID

(57) Abstract

The present invention provides a pharmaceutical composition for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy, comprising ALA or a precursor therefor, together with at least one surface penetration assisting agent, and optionally one or more chelating agents whereby therapeutic efficacy of the ALA is enhanced.

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Photochemotherapeutic compositions containing 5-aminolevulinic acid

The present invention relates to pharmaceutical compositions for use in the treatment of disorders or abnormalities of the skin and other body surfaces by photochemotherapy.

Abnormalities or disorders, such as neoplasms or psoriatic plaques, of the skin or other epithelial organs or mucosa are conventionally treated by surgery, radiotherapy, cryotherapy or chemotherapy. These treatments however, often have significant and serious drawbacks such as toxicity, carcinogenicity, or other unpleasant side effects or general discomfort resulting from the treatment.

More recently photochemotherapy has been proposed for the treatment of certain skin cancers and psoriasis. This involves the application of photosensitizing agents which are activated by exposure to light i.e. the active, e.g. cytotoxic form, of the drug is formed at the site of application upon exposure to light. A range of photosensitizing agents are known, including notably the psoralens, the porphyrins, the chlorins and the phthalocyanins. Such drugs become toxic when exposed to light.

Photosensitizing drugs may exert their effects by a variety of mechanisms, directly or indirectly. Thus for example, certain photosensitizers become directly toxic when activated by light, whereas others act to generate toxic species, e.g. oxidising agents such as singlet oxygen or other oxygen-derived free radicals, which are extremely destructive to cellular material and biomolecules such as lipids, proteins and nucleic acids. Psoralens are an example of directly acting photosensitizers; upon exposure to light they form adducts and cross-links between the two strands of DNA molecules, thereby inhibiting DNA synthesis. The

unfortunate risk with this therapy is that unwanted mutagenic and carcinogenic side effects may occur.

This disadvantage may be avoided by selecting photosensitizers with an alternative, indirect mode of action. For example porphyrins, which act indirectly by generation of toxic oxygen species, have no mutagenic side effects and represent more favourable candidates for photochemotherapy.

One such porphyrin-based drug, Photofrin, is presently being tested as a photosensitizer in cancer therapy. Its main disadvantage is that since it must be administered parenterally, generally intravenously, it causes photosensitization of the skin which may last for several weeks following i.v. injection; Photofrin consists of large oligomers of porphyrin and it does not readily penetrate the skin when applied topically.

Alternative porphyrin-based photosensitizers have been investigated. Porphyrins are naturally occurring precursors in the synthesis of heme. In particular, heme is produced when iron (Fe^{3+}) is incorporated in protoporphyrin IX (Pp) by the action of the enzyme ferrochelatase. Pp is an extremely potent photosensitizer, whereas heme has no photosensitizing effect.

A so-called "hematoporphyrin derivative" (Hpd) has also been reported for use in cancer photochemotherapy (see for example S. Dougherty. J. Natl. Cancer Ins., 1974, 52; 1333; Kelly and Snell, J. Urol, 1976, 115 : 150). Hpd is a complex mixture obtained by treating haematoporphyrin with acetic and sulphuric acids, after which the acetylated product is dissolved with alkali. Clearly, there are disadvantages in using an undefined mixture as a drug. Moreover since Hpd must also be administered by injection, it suffers from the same type of undesirable photosensitization drawback as does Photofrin.

More recently, a Pp precursor, 5-aminolevulinic

acid (ALA) has been investigated as a photochemotherapeutic agent for certain skin cancers. ALA, which is formed from succinyl CoA and glycine in the first step of heme synthesis, is to a limited extent able to penetrate the skin and lead to a localised build-up of Pp; since the action of ferrochelatase (the metallating enzyme) is the rate limiting step in heme synthesis, an excess of ALA leads to accumulation of Pp, the photosensitizing agent. Thus, by applying ALA topically to skin tumours, and then after several hours exposing the tumours to light, a beneficial photochemotherapeutic effect may be obtained. Since the skin covering basilomas and squamous cell carcinomas is more readily penetrated by ALA than healthy skin, and since the concentration of ferrochelatase is low in skin tumours, it has been found that topical application of ALA leads to a selectively enhanced production of Pp in tumours.

However, for a number of reasons photochemotherapy with ALA is not entirely satisfactory. Firstly, ALA is not able to penetrate tumours and other tissues with sufficient efficacy, for example to enable treatment of a wide range of tumours or other conditions. A second major disadvantage is the instability of ALA in pharmaceutical formulations. The present invention addresses these problems.

In particular, the present invention aims to provide photochemotherapeutic compositions which are more stable, better able to penetrate the tumour or other abnormality, and which have an enhanced photochemotherapeutic effect over those described in the prior art.

In one aspect, the present invention provides a pharmaceutical composition for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy, comprising ALA or a precursor

therefor, together with at least one surface penetration assisting agent and optionally one or more chelating agents whereby therapeutic efficacy of the ALA is enhanced.

Alternatively viewed, the invention can be seen to provide the use of ALA or a precursor therefor together with at least one surface penetration assisting agent in the preparation of a composition for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

The term "precursors" as used herein refers to precursors for ALA which are converted metabolically to ALA and are thus essentially equivalent to ALA.

The surface-penetration assisting agent may be any of the skin-penetration assisting agents described in the pharmaceutical literature e.g. DMSO and other dialkylsulphoxides, in particular n-decylmethyl-sulphoxide (NDMS), dimethylsulphacetamide, dimethylformamide (DMFA), dimethylacetamide, glycols, various pyrrolidone derivatives (Woodford et al., J. Toxicol. Cut. & Ocular Toxicology, 1986, 5: 167-177), and Azone® (Stoughton et al., Drug Dpv. Ind. Pharm. 1983, 9: 725-744), or mixtures thereof.

DMSO however has a number of beneficial effects and is strongly preferred. Thus, in addition to the surface-penetration assisting effect (DMSO is particularly effective in enhancing the depth of penetration of the active agent into the tissue), DMSO has anti-histamine and anti-inflammatory activities, leading to a reduction in pain during the light exposure process. In addition, DMSO has been found to increase the activity of the enzymes ALA-synthase and ALA-dehydrogenase (the enzymes which, respectively, form and condense ALA to porphobilinogen) thereby enhancing the formation of the active form, Pp.

However, in certain conditions such as psoriasis,

the lesions are relatively easily penetrated and the penetrating agent may be less beneficial. In some circumstances, for example in the case of skin cancers where the lesions are difficult to penetrate, the surface penetration assisting agent may be applied in a preliminary step, generally at a higher concentration.

Thus, the various active components need not be applied simultaneously within the same composition, but may, according to clinical need, be administered separately and sequentially. Indeed, it has been observed that in many cases a particularly beneficial photochemotherapeutic effect may be obtained by pre-treatment with the surface-penetration assisting agent in a separate step, prior to administration of the ALA or its precursor. Furthermore, in some situations a pre-treatment with the surface-penetration assisting agent, followed by administration of the photochemotherapeutic agent in conjunction with the surface-penetration assisting agent has been found to be beneficial. If such a pre-treatment step is employed, the photochemotherapeutic agent may subsequently be administered up to several hours following pre-treatment eg. at an interval of 5-60 minutes following pre-treatment.

Viewed from a further aspect, the invention thus provides a product comprising ALA or a precursor therefor, together with at least one surface-penetration assisting agent, and optionally one or more chelating agents as a combined preparation for simultaneous, separate or sequential use in treating disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

Alternatively viewed, this aspect of the invention also provides a kit for use in photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

a) a first container containing ALA or a precursor therefor;

b) a second container containing at least one surface penetration assisting agent; and optionally

c) one or more chelating agents contained either within said first container or in a third container.

Chelating agents are optionally contained in the pharmaceutical composition of the invention for two effects, firstly to enhance the stability of ALA and secondly to enhance its accumulation. The latter effect is achieved by the chelation of iron, thereby preventing the inactivating action of ferrochelatase in incorporating the metal into Pp, leading to Pp build-up. The photosensitizing effect is thus enhanced.

Aminopolycarboxylic acid chelating agents are particularly suitable for use in this regard, including any of the chelants described in the literature for metal detoxification or for the chelation of paramagnetic metal ions in magnetic resonance imaging contrast agents. Particular mention may be made of EDTA, CDTA (cyclohexane diamine tetraacetic acid), DTPA and DOTA. EDTA is preferred, especially for the stabilisation of ALA. To achieve the iron-chelating effect, desferrioxamine and other siderophores may also be used, e.g. in conjunction with aminopolycarboxylic acid chelating agents such as EDTA.

A preferred composition or product according to the invention, comprises ALA, DMSO, EDTA and desferrioxamine.

The concentration of ALA in the composition is preferably in the range 10 to 30%, e.g. 15 to 25%; the concentration of chelating agent is preferably in the range 1 to 10% e.g. about 2.5%; the concentration of surface penetration assisting agent, e.g. DMSO, is preferably in the range 2 to 50% e.g. about 10%. All

percentages stated above are by weight. However, as mentioned above, where the surface penetration assisting agent is administered separately in a preliminary step, it may be applied at higher concentrations, even up to 100%.

The abnormalities and disorders which may be treated according to the present invention include any malignant, pre-malignant and non-malignant abnormalities or disorders responsive to photochemotherapy eg. tumours or other growths, skin disorders such as psoriasis or actinic keratoses, skin abrasions, and other diseases or infections eg. bacterial, viral or fungal infections, for example Herpes virus infections. The invention is particularly suited to the treatment of diseases, disorders or abnormalities where discrete lesions are formed to which the compositions may be directly applied (lesions is used here in a broad sense to include tumours and the like).

The internal and external body surfaces which may be treated according to the invention include the skin and all other epithelial and serosal surfaces, including for example mucosa, the linings of organs eg. the respiratory, gastro-intestinal and genito-urinary tracts, and glands with ducts which empty onto such surfaces (e.g. liver, sebaceous glands, mammary glands, salivary glands and seminal vesicles). In addition to the skin, such surfaces include for example the lining of the vagina, the endometrium and the urothelium. Such surfaces may also include cavities formed in the body following excision of diseased or cancerous tissue eg. brain cavities following the excision of tumours such as gliomas.

Exemplary surfaces thus include: (i) skin and conjunctiva; (ii) the lining of the mouth, pharynx, oesophagus, stomach, intestines and intestinal appendages, rectum, and anal canal; (iii) the lining of the nasal passages, nasal sinuses, nasopharynx, trachea,

bronchi, and bronchioles; (iv) the lining of the ureters, urinary bladder, and urethra; (v) the lining of the vagina, uterine cervix, and uterus; (vi) the parietal and visceral pleura; (vii) the lining of the peritoneal and pelvic cavities, and the surface of the organs contained within those cavities; (viii) the dura mater and meninges; (ix) any tumors in solid tissues that can be made accessible to photoactivating light e.g. either directly, at time of surgery, or via an optical fibre inserted through a needle.

The compositions of the invention may be formulated in conventional manner optionally with one or more physiologically acceptable carriers or excipients, according to techniques well known in the art. Topical compositions are preferred, especially when all active components are to be administered together and include gels, creams, ointments, sprays, lotions, salves, sticks, soaps, powders, pessaries, aerosols, drops and any of the other conventional pharmaceutical forms in the art.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will, in general, also contain one or more emulsifying, dispersing, suspending, thickening or colouring agents. Powders may be formed with the aid of any suitable powder base. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing, solubilising or suspending agents. Aerosol sprays are conveniently delivered from pressurised packs, with the use of a suitable propellant.

Alternatively, the surface penetration assisting agent is applied topically in a separate step, and the ALA, optionally together with one or more chelating agents may be administered by an alternative route e.g.

orally or parenterally for example by intradermal, subcutaneous, intraperitoneal or intravenous injection. Alternative pharmaceutical forms thus include plain or coated tablets, capsules, suspensions and solutions containing the active components optionally together with one or more inert conventional carriers and/or diluents, e.g. with corn starch, lactose, sucrose, microcrystalline cellulose, magnesium stearate, polyvinylpyrrolidone, citric acid, tartaric acid, water, water/ethanol, water/glycerol, water/sorbitol, water/polyethyleneglycol, propyleneglycol, stearylalcohol, carboxymethylcellulose or fatty substances such as hard fat or suitable mixtures thereof.

Following administration to the surface, the area treated is exposed to light to achieve the photochemotherapeutic effect. The length of time following administration, at which the light exposure takes place will depend on the nature of the composition but will generally be less than when using ALA alone. This can generally be in the order of 0.5 to 48 hours, e.g. 1 to 3 hours.

The irradiation will in general be applied at a dose level of 40 to 100 Joules/cm^2. At 100 Joules/cm^2, penetration of the radiation is found to be relatively deep. Using a combination of ALA, DMSO and EDTA, the irradiation dose may be as much as 40% less than that required using ALA alone.

In a further aspect of the invention, we have also surprisingly found that the wavelength of light used for irradiation is important in achieving an efficaceous photochemotherapeutic effect. Conventionally, when porphyrins are used in photochemotherapy they are irradiated with light at about the absorption maximum of the porphyrin. Thus, for example in the case of the prior art use of ALA in photochemotherapy of skin cancer, wavelengths in the region 350-640 nm, preferably 610-635 nm were employed. We have found however that by

selecting a broad range of wavelengths for irradiation, extending beyond the absorption maximum of the porphyrin, the photosensitizing effect may be enhanced. Whilst not wishing to be bound by theory, this is thought to be due to the fact that when Pp, and other porphyrins, are exposed to light having wavelengths within its absorption spectrum, it is degraded into various photo-products including in particular photoprotoporphyrin (PPp). PPp is a chlorin and has a considerable photo-sensitizing effect; its absorption spectrum stretches out to longer wavelengths beyond the wavelengths at which Pp absorbs ie. up to almost 700 nm (Pp absorbs almost no light above 650 nm). Thus in conventional photochemotherapy, the wavelengths used do not excite PPp and hence do not obtain the benefit of its additional photosensitizing effect. Irradiation with wavelengths of light in the range 500-700 nm has been found to be particularly effective. It is particularly important to include the wavelengths 630 and 690 nm.

A further aspect of the invention thus provides a method of photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising administering to the affected surfaces, a composition or product as hereinbefore defined, and exposing said surfaces to light, preferably to light in the wavelength region 500-700 nm.

Methods for irradiation of different areas of the body, eg. by lamps or lasers are well known in the art (see for example Van den Bergh, Chemistry in Britain, May 1986 p. 430-439).

The invention will now be described in more detail in the following non-limiting Examples, with reference to the drawings in which:

Figure 1 is a graph showing the averaged results for 5-ALA penetration through tissue biopsy segments of BCC lesions, achieved using three different ALA

formulations as described in Example 4 (—▲— 5-ALA; —□— 5-ALA, DMSO, EDTA; —◆— 5-ALA, DMSO, EDTA with DMSO pre-treatment); abscissa shows segment number; ordinate shows fluorescence intensity (units?);

Figure 2 shows the results of BCC phototherapy after topical application of 5-ALA composite cream. Application time - 4-5 hours, dose of irradiation - 100 J/cm². S - clinically superficial lesions (17), UN - ulcerated nodular lesions (31), ■PR - partial response, □CR - complete response;

Figure 3 shows the results of SCC phototherapy after topical application of 5-ALA composite cream. Application time - 4-5 hours, dose of irradiation - 100 J/cm². S - clinically superficial lesions (5), UN - ulcerated nodular lesions (2), ■PR - partial response, □CR - complete response.

Example 1

Formulation

An ALA-containing cream, containing 5-30% ALA, is prepared by admixing ALA with a commercially available cream base.

A 20% ALA cream was prepared by admixture with "Urguentum Merck" cream base (available from Merck) consisting of silicon dioxide, paraffin liq., vaseline, album, cetostearol., polysorbat. 40, glycerol monostearate, Miglyol®812 (a mixture of plant fatty acids), polypropyleneglycol., and purified water.

Example 2

The effects of DMSO in enhancing the photochemotherapeutic effect of ALA were investigated.

A cream containing 20% ALA, prepared as described in Example 1, was applied to ulcero-nodular lesions of basal cell carcinomas on patients. Photo dynamic therapy (PDT) was then applied using standard conditions (630nm, 100 Joules/cm^2). The results are shown in Table 1. In this study about 33% response was achieved.

Table 1 - ALA Light 630nm, 100 joules/cm2

Type of lesion	Number of un. lesions (Total No. of lesions)	Complete response number	%	Partial response (>50% reduction) number	%
Ulcero-nodular (u.n)	4 (94)	1	25%	3	75%
Ulcero-nodular (u.n)	29 (225)	10	34%	19	66%

Subsequently, the study was repeated using DMSO either as a pre-treatment, applied at a concentration of 50-96% for 15 minutes, and/or as a co-factor included in the ALA-containing cream at a concentration of 2-4%, as well as in a cream also containing EDTA (2-4%). The results are shown in Table 2, and indicate an improved cure rate up to about 60% (as compared with Table 1).

Table 2 - ALA + DMSO + EDTA - Light 630nm, 40-100 joules/cm2

Type of lesion	Number of u.n lesions (total number of lesions)	Complete response number	%	Partial response (>50% reduction) number	%	Deficient response (<50% reduction) number	%
Ulcero-nodular (u.n)	42 (219)	24	57%	12	29%	6	14%
Ulcero-nodular (u.n)	42 (234)	24	57%	12	29%	6	14%

Example 3

In this study the penetration effect on lesions, with or without DMSO pre-treatment, was assessed by measuring fluorescence intensity as follows.

Neoplastic and adjacent normal skin tissue samples from patients with basal cell carcinomas (superficial and nodular) were removed before and at different time intervals after topical application of ALA alone or ALA plus laser irradiation. The samples were immediately immersed in liquid nitrogen and the tissue sections were then cut with a cryostat microtome. The localization pattern of the ALA-induced porphyrin fluorescence in the sections was directly observed by means of fluorescence microscopy. The same frozen sections were subsequently stained with routine H&E staining for histological identification. Fluorescence microscopy was carried out with an Axioplan microscope (Zeiss, Germany). The

fluorescence images and quantitative measurements were performed by a CCD camera (Astromed CCD 3200, Cambridge, UK) and an image processing unit (Astromed/Visilog, PC 486DX2 66 MHz VL).

ALA-containing cream as described in Example 1, additionally containing EDTA, was applied topically for 3 hours, with or without a preliminary DMSO pre-treatment step. The results are shown in Table 3 and clearly demonstrate a significantly better penetration following pre-treatment with DMSO.

Table 3

The ALA-induced fluorescence intensity of the nodular BCC lesions with or without DMSO treatment followed by topical application of ALA-cream containing DMSO and EDTA for 3 hours is compared as follows:

	Fluorescence intensity[a]	
Depth[b]	with DMSO pre-treatment	without DMSO pre-treatment
background[c]	1	1
epidermis	1.24	1.1
1	2.11	1.21
2	1.53	0.84
3	1.56	
4	1.76	
5	1.77	
6	1.68	
7	1.36	
8	1.24	

[a] Data on fluorescence intensity (mean values of counts per pixel) are normalized to 1 for background.

[b] Whole depth of BCC lesion (from epidermis to scale 8) is about 3.5 mm.

[c] Background is mostly due to the auto-fluorescence emitted from normal fibro-connective tissue in dermis. Thus, there is a much lower auto-fluorescence background in BCC lesion than normal dermis.

Example 4

A series of experiments have been carried out comparing the actual penetration of (1) 5-ALA (20% concentration in carrier cream), (2) 5-ALA with 4% EDTA and 20% DMSO, and (3) 5-ALA with 4% EDTA and 20% DMSO and with 15-30 minutes of pretreatment with DMSO.

Experimental details

5-ALA

5-Amino levulinic acid (5-ALA) hydrochloride was purchased from Porphyrine Products, Logan, Utah, USA. All the 5-ALA used in the experiments was accompanied by a Certificate of Analysis stating the identity and the purity of the chemical. 5-ALA was stored in the dark (in a refrigerator).

The Cream

The cream, containing 20% ALA (w/v) was produced by careful mixing of the ingredients as follows:

To produce 5 grams of the cream, 1 gram of 5-ALA, 1 gram of distilled and purified water and 3 grams of Unguentum Merck was mixed. The DMSO and/or EDTA used in the cream were included by reducing the corresponding amount of Unguentum Merck. The cream was kept at 4°C, in the dark, until used, usually within one week.

Biopsies were acquired from patients being treated with these cream composition and subsequently analyzed by fluorescence measurements as follows.

Biopsies

Neoplastic and adjacent normal skin tissue samples from patients with basal cell carcinomas (BCC) (superficial and nodular) were removed before and at different time intervals after topical applications of 5-ALA cream alone or 5-ALA cream plus laser irradiation. The samples were immediately immersed in liquid nitrogen and the tissue sections were then cut with a cryostat microtome. The localization pattern of the ALA-induced porphyrin fluorescence in the sections was directly observed by means of fluorescence microscopy. The same frozen sections were subsequently stained with routine H&E staining for histological identification. Fluorescence microscopy was carried out with an Axioplan microscope (Zeiss, Germany). The fluorescence images and quantitative measurements were performed by a CDD camera (Astromed CCD 3200, Cambridge, UK) and an image processing unit (Astromed/Visilog, PC 486DX2 66 MHz VL).

Selection of lesions

All lesions selected for these studies were histological or cytological verified BCC lesions.

Data were collected for epidermis and for underlying segments. Segments are defined as the segments of the BCC lesions from superficial layer to deep dermis. The depth (i.e. thickness) of each segment was about 0.2-0.3 mm. Data were collected for segments as long as fluorescence was observed.

RESULTS

The results for the analysis of the three optional treatments are given below:

(1) 5-ALA

The fluorescence data for 3 different cases using 5-ALA in a 20% concentration in a commercial carrier cream are given in Table 4. The table reveals that measurable penetration is limited to maximum 3 segments. The table also gives the average data in each segment for the three cases.

(2) 5-ALA with additives (EDTA & DMSO)

The individual fluorescence data for 4 different cases using 5-ALA in a 20% concentration in a commercial carrier cream, 4% EDTA and 20% DMSO are given in Table 2. The data for each segment for the 4 cases are also averaged in Table 5. The Table shows that the concentration of 5-ALA measured as fluorescence intensity is about twice what was observed for 5-ALA alone and that the penetration is deeper. Even at the fourth segment, the average fluorescence measurement data is higher than the highest value observed in the experiments with 5-ALA alone (Table 4).

(3) 5-ALA with additives and pretreatment

Pretreatment of the skin with DMSO for 15-30 minutes before application of the cream enhances the penetration of 5-ALA in skin. This has been demonstrated by a series of 5 cases of topical applications of 5-ALA with DMSO. The results for these cases are shown both individually and averaged in Table 6 and reveal up to a three-fold increase in penetration. Furthermore, the

fluorescence intensity (and the 5-ALA concentration) is significantly higher than that for the use of 5-ALA alone.

Comparison of data

The averaged results from each segment for the individual cases in the three treatment modes show clearly an increased skin penetration when using DMSO. This is illustrated in Figure 1.

As shown in this figure, the fluorescence intensity is higher when DMSO is used in the cream. Furthermore, the figure shows that using pretreatment with DMSO, both the fluorescence intensity and the penetration depth increases significantly.

CONCLUSION

The fluorescence data given here shows that DMSO in cream enhances the uptake of 5-ALA, generally with a factor of 2. Furthermore, pretreatment combined with the use of 5-ALA with additives increases both penetration and uptake a factor of more than 4 compared with the untreated cases.

Table 3 - Fluorescence measurements of nodular BCC. Topical application of 5-ALA.

Case	1	2	3	Average
Segment				
1	504	68	170	247
2	377	0	78	152
3	116	0	29	48

Table 4 - Fluorescence measurements of nodular BCC. Topical application of 5-ALA (20%)/4% EDTA/20% DMSO

Case	1	2	3	4	Average
Segment					
1	130	485	623	675	478
2	0	714	365	353	358
3		637	0	303	235
4		499		159	165

Table 5 - Fluorescence measurements of nodular BCC. Topical application of 5-ALA (20%)/4% EDTA/20% DMSO and 15-20 minuntes pretreatment

Case	1	2	3	4	5	Average
Segment						
1	878	439	809	1152	827	821
2	535	573	864	1624	170	753
3	184	652	922	1229	24	602
4	0	823	0	816	0	328
5		604		859		293
6		587		763		270
7		234		904		228

Example 5

MATERIALS AND METHODS

Patients

Fifty five ambulatory patients of the Plastic Surgery Clinic, Sheba Medical Center, Tel Hashomer, took part in these clinical trials. Forty eight patients had basal

cell carcinoma (BCC) and 7 patients had squamous cell carcinoma (SCC) of the skin. Seventeen of the BCC lesions were identified as clinically superficial and 31 as ulcerated nodular BCC. Of the 7 patients with SCC, 5 of them had superficial lesions and 2 had ulcerated nodular. The superficial lesions appeared flat and not ulcerated, but on histological examination were found to be deeply penetrating. A routine clinical evalutaion, including biopsy, was performed during the 3 month interval following the treatment. The results of the treatment were evalutated according to the following criteria: complete response (CR, i.e. clinical tumor disappearance); partial response (PR, i.e. more than 50% reduction of tumor size) and no response (NR, i.e. less than 50% reduction of tumor size).

Chemicals

5-ALA was purchased from Sigma, St. Louis, Mo. Two formulas of 5-ALA cream were used. Formula A contained 20% of 5-ALA in a base cream (Decoderm, Merck, Germany), and formula B, the 5-ALA composite cream, contained an additional 2% of DMSO and 2% of EDTA disodium salt (Titriplex III, Merck, Germany). The cream was prepared daily just prior to application and applied onto the lesion after cleaning the area with a saline solution. Approximately 0.2 ml of the cream was applied to 1cm^2 of skin area. After the cream application, the skin area was covered by a plastic adhesive dressing (Tegaderm) and an aluminum foil shield for protection from light exposure. The cream was left on the skin for 2 - 5 hours. At the end of these periods, most of the cream was not absorbed into the skin; it was removed before fluorescence measurement and applied again for continuation of the treatment.

Fluorescence measurements

After the cream application, production of protoporphyrin (PP) was measured *in situ* by a laser-induced fluorescence (LIF) method. Porphyrin production in the skin lesions was measured every hour by the LIF method. The fluorescence was measured directly, *in situ*, by a bundle of optic fibers that simultaneously delivered excitation light and transferred the red emitted light to the spectrofluorimeter. A 502 nm line of Ar^+ laser (Coherent, Palo Alto, CA, model Innova 200) was used for fluorescence excitation of ALA-induced PP. The laser light was passed through an interference filter and transferred to the sample via one of the legs of a bifurcated fiber bundle (Oriel, Stratford, CT, model 77533). The common end tip of this bundle was fixed at a distance of 8mm from the object to form a light spot of about 6mm in diameter. The beam power on the sample surface was measured with a laser power meter (Ophir, Israel, model PD2-A), and was 15mW. The fluorescence measurements were performed on a digital fluorimeter (Perkin-Elmer, Norwalk, CT, model LS-50B). For this purpose, the end tip of a second leg of the bundle was placed in front of the entrance slit of the fluorimeter with a longpass filter (Schott, Germany, type OG 550). The filter was used for transmission of the luminescence signal and for blocking the laser light excitation. Time of a fluorescence spectrum recording was 10 sec, and the average of several recordings was used for each kinetic data point.

Histological examination of skin biopsies

A biopsy was taken several weeks prior to 5-ALA application, and again 2 - 3 months after photodynamic procedure. Each biopsy was sectioned, stained with Hematoxylin and Eosin and histologicaly examined. The

results of the histological examination were compared with clinical observations.

Light irradiation

The light delivery system VERSA-LIGHT (Medic Lightech Ltd, Technion, Haifa, Israel) was used for tumor photoradiation. Red light in the range of 600 to 720 nm was transmitted through an optical fiber. At the tip of the fiber the light power measured by the power meter (Coherent, model 210) was 1.7 W. During the photoirradiation, the fiber was maintained in a stationary position. The light dose for each lesion was $100J/cm^2$.

RESULTS

The PP production in BCC and SCC lesions after topical application of the 5-ALA and 5-ALA composite creams was detected in situ by the LIF method. The characteristic PP fluorescence spectra with peaks at 635 and 704 nm were registered after application of the two 5-ALA cream formulations. A typical porphyrin fluorescence measurement in a patient with multiple BCC treated with 5-ALA-cream is shown in Table 7. On the same patient, 4 lesions were treated with 5-ALA cream and 5 lesions were treated with 5-ALA composite cream. The intensity of PP fluorescence in the tumors was time-dependent and cream composition-dependent. The PP accumulation in the tissues was detected after 2 hours and was maximal after 4 hours of cream exposure.

Table 7. In situ fluorescence intensity (635 nm) of 5-ALA-induced PP in a patient with multiple BCC.

Area	Cream	Fluorescence intensity (a.u.)			
		Time of application (hr)			
		2	3	4	5
1. Back	5-ALA	60			215
2. Hand	5-ALA		70	100	
3. Hand	5-ALA		44	46	
4. Hand	5-ALA		80	130	
Average		**60**	**65**	**92**	**215**
5. Neck	5-ALA comp	93		355	
6. Arm	5-ALA comp	154		340	
7. Arm	5-ALA comp		200		290
8. Leg	5-ALA comp		190		350
9. Leg	5-ALA comp		205		400
Average		**124**	**198**	**348**	**347**

The results of fluorescence measurement clearly indicate that PP accumulation induced by the 5-ALA composite cream was markedly higher than that induced by the 5-ALA cream. The differences were observed after a 2-hour exposure, and increased with time. After 3 hours, PP fluorescence in the tumors treated by the composite cream was 300% higher than that treated by the simple cream. On the basis of these results, the 5-ALA composite cream has been chosen for clinical PDT use for BCC and SCC patients and the time interval of the cream application is 4-5 hours.

Figure 2 shows the treament results of 48 patients with BCC after topical application of 5-ALA composite cream. Clinically superficial BCC tumors were very responsive to PDT, and the 17 lesions treated showed a 100% CR. The overall result in the 48 patients showed an 85.4% CR. After PDT, an initial edematous reaction was observed, followed by necrosis of the tumor tissue. Histological examination showed complete disappearance of the tumor. Seven out of these 48 patients were evaluated as PR, and experienced tumor regrowth. None of the lesions was considered as non-responsive to the treatment.

SCC patients were similarly treated with the 5-ALA composite cream. Figure 3 summarizes the results of the treatment. The 5 superficial SCC had a 100% CR to PDT. Histological examination showed disappearance of the tumors after 3 months from the date of PDT. The ulcerated nodular lesions had a PR to the treatment.

CONCLUSION

In the present study two parameters were investigated in order to improve the efficiency of the 5-ALA treatment of human malignant skin tumors: a) enhancement of 5-ALA penetration into the tissues and b) enhancement of porphyrin production in the tumors. For these purposes, DMSO and EDTA were used in combination with 5-ALA.

A novel 5-ALA cream for in vivo application has been developed which contains 5-ALA, DMSO and EDTA. The LIF method has been used clinically for in situ PP production examination in tumors in order to determine the optimal cream formulation and time interval for application. The results clearly indicate that PP in situ fluorescence intensity in the tumors is higher when the 5-ALA is applied with DMSO. The present results

reveal a marked advantage of the composite 5-ALA cream containing DMSO and EDTA in human skin carcinomas. Maximal PP accumulation in tumors was observed 4 - 5 hours after cream application, and this is the optimal time for photoirradiation of the lesions. The clinical results point to the high potential for using PDT with topical 5-ALA composite cream application for treatment of BCC and SCC lesions. CR was observed in 85.4% cases of BCC and 100% cases of superficial SCC, with no side effects.

Claims

1. A pharmaceutical composition for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy, comprising ALA or a precursor therefor, together with at least one surface penetration assisting agent.

2. A product comprising ALA or a precursor therefor, together with at least one surface-penetration assisting agent, as a combined preparation for simultaneous, separate or sequential use in treating disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

3. A compostion as claimed in claim 1 or a product as claimed in claim 2, wherein the surface penetration assisting agent is a dialkylsulphoxide.

4. A composition or product as claimed in any one of claims 1 to 3, wherein the surface penetration assisting agent is dimethylsulphoxide (DMSO).

5. A composition or product as claimed in any one of claims 1 to 4, further comprising one or more chelating agents.

6. A composition or product as claimed in claim 5, further comprising an aminopolycarboxylic acid chelating agent.

7. A composition or product as claimed in claim 6, wherein the aminopolycarboxylic acid chelating agent is EDTA.

8. A composition or product as claimed in any one of claims 1 to 7, wherein the concentration of ALA is 10 to 30% by weight.

9. A composition or product as claimed in any one of claims 1 to 8, wherein the concentration of the surface-penetration assisting agent is 2 to 50% by weight.

10. A composition or product as claimed in any one of claims 1 to 9, wherein the concentration of the chelating agent is 1 to 10% by weight.

11. A composition or product as claimed in any one of claims 1 to 10, in a form suitable for topical administration.

12. The use of ALA or a precursor therefor together with at least one surface penetration assisting agent in the preparation of a composition for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

13. Use as claimed in claim 12, wherein said composition further comprises one or more chelating agents.

14. A kit for use in photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

a) a first container containing ALA or a precursor therefor; and
b) a second container containing at least one surface penetration assisting agent.

15. A kit as claimed in claim 14, further comprising one or more chelating agents contained either within said first container or in a third container.

16. A method of photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising administering to the affected surfaces, a composition or product as defined in any one of claims 1 to 11, and exposing said surfaces to light.

17. A method as claimed in claim 16, wherein said surfaces are exposed to light in the wavelength region 500-700nm.

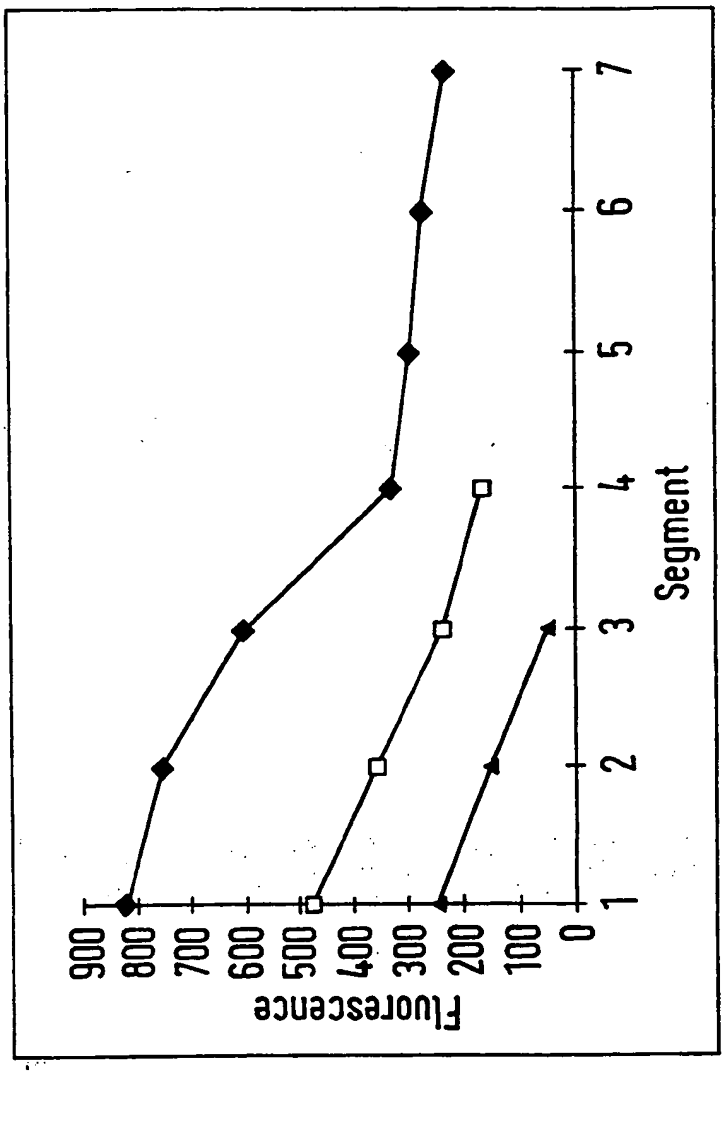
Fluorescence
900
800
700
600
500
400
300
200
100
0
Segment
1
2
3
4
5
6
7

FIG.1

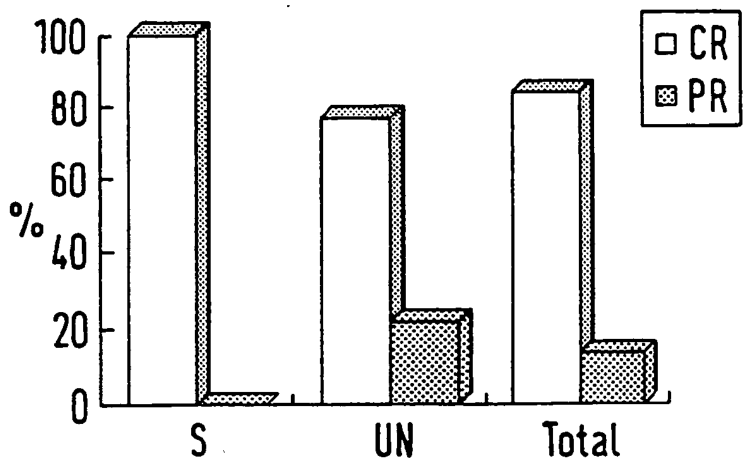

FIG. 2

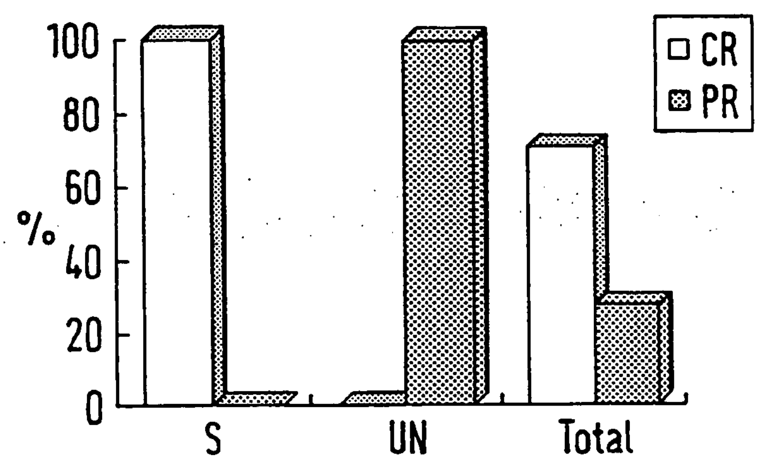

FIG. 3

INTERNATIONAL SEARCH REPORT

Internat . Application No
PCT/GB 94/01951

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/195 A61K47/20 A61K47/18 A61K41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,X	PROCEEDINGS OF PHOTODYNAMIC THERAPY OF CANCER, vol.2078, 4 September 1993, BUDAPEST pages 355 - 362 MALIK Z. ET AL 'INDUCTION OF PROTOPORPHYRIN BIOSYNTHESIS AND PHOTODINAMYC INACTIVATION OF B16 MELANOMA CELLS' see page 356, paragraph 2.2 --- -/--	1-4,9, 11,12, 14,16,17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
20 January 1995	31. 01. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Boulois, D

Intern. ·al Application No
PCT/GB 94/01951

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	BIOCHEM. PHARMACOL., vol.42, no.6, 1991 pages 1307 - 13 DOOSTDAR H. ET AL 'THE EFFECT OF DIMETHYLSULFOXIDE AND 5-AMINOLEVULINIC ACID ON THE ACTIVITY OF CYTOCHROME P-450-DEPENDENT MIXED FUNCTION OXIDASE AND UDP-GLUCURONOSYL TRANSFERASE ACTIVITIES IN HUMAN HEP G2 HEPATOMA CELLS' see page 310; figure 2A	1-4 5-7
Y	CANCER LETTERS, vol.65, 1992 pages 127 - 131 HANANIA J. ET AL 'THE EFFECT OF EDTA AND SERUM ON ENDOGENOUS PORPHYRIN ACCUMULATION AND PHOTODINAMYC SENSITIZATION OF HUMAN K562 LEUKEMIC CELLS' see page 128, paragraph 3	5-7
A	WO,A,91 01727 (QUEEN'S UNIVERSITY AT KINGSTON) 21 February 1991 see page 6, line 13 - line 24 see page 7, line 19 - page 8, line 6 see page 11 - page 12; example 1	1-17
A	WO,A,90 06748 (FMC CORPORATION) 28 June 1990 see page 11 - page 14; example 1	1-17
A	CHEMICAL ABSTRACTS, vol. 114, no. 11, 18 March 1991, Columbus, Ohio, US; abstract no. 96819, MAMADALIEV A.S. ET AL 'COMPOSITION FOR DEFOLIATION OF COTTON CONTAINIG IPYRIDIUM SALT' see abstract & SU,A,1 598 944 (INSTITUTE OF GENERAL PHYSICS, ACADEMY OF SCIENCES, USSR) 15 October 1990	1-17
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,P, X	PROCEEDINGS OF OPTICAL METHODS FOR TUMOR TREATMENT AND DETECTION: MECHANISMS AND TECHNIQUES IN PHOTODINAMYC THERAPY III, vol.2133, 23 January 1994, LOS ANGELES pages 194 - 199 DOUGHERTY T. ET AL 'PHOTOFRIN AND 5-AMINOLEVULINIC ACID PERMEATION THROUGH ORAL MUCOSA IN VITRO' see the whole document	1-17

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Information on patent family members

International Application No
PCT/GB 94/01951

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SU-A-1598944	15-10-90	NONE		